Biochemical and Biophysical Research Communications 437 (2013) 573–578

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Saturated fatty acid palmitate induces extracellular release of histone H3: A possible mechanistic basis for high-fat diet-induced inflammation and thrombosis

Chandan Shrestha [a,b], Takashi Ito [a], Ko-ichi Kawahara [c], Binita Shrestha [b], Munekazu Yamakuchi [b], Teruto Hashiguchi [b], Ikuro Maruyama [a,*]

[a] Department of Systems Biology in Thromboregulation, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan
[b] Department of Laboratory and Vascular Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan
[c] Department of Biomedical Engineering, Osaka Institute of Technology, Osaka, Japan

ARTICLE INFO

Article history:
Received 18 June 2013
Available online 10 July 2013

Keywords:
High-fat diet
Palmitate
ROS
JNK
Histone H3
Inflammation

ABSTRACT

Chronic low-grade inflammation is a key contributor to high-fat diet (HFD)-related diseases, such as type 2 diabetes, non-alcoholic steatohepatitis, and atherosclerosis. The inflammation is characterized by infiltration of inflammatory cells, particularly macrophages, into obese adipose tissue. However, the molecular mechanisms by which a HFD induces low-grade inflammation are poorly understood. Here, we show that histone H3, a major protein component of chromatin, is released into the extracellular space when mice are fed a HFD or macrophages are stimulated with the saturated fatty acid palmitate. In a murine macrophage cell line, RAW 264.7, palmitate activated reactive oxygen species (ROS) production and JNK signaling. Inhibitors of these pathways dampened palmitate-induced histone H3 release, suggesting that the extracellular release of histone H3 was mediated, in part, through ROS and JNK signaling. Extracellular histone activated endothelial cells toexpress the adhesion molecules ICAM-1 and VCAM-1 and the procoagulant molecule tissue factor, which are known to contribute to inflammatory cell recruitment and thrombosis. These results suggest the possible contribution of extracellular histone to the pathogenesis of HFD-induced inflammation and thrombosis.

1. Introduction

Overweight status and obesity are risk factors for the development of insulin resistance, type 2 diabetes mellitus, atherosclerosis, and fatty liver diseases [1], which are generally caused by increased consumption of high-fat foods with reduced physical activity [2]. Beside these metabolic disorders, obesity is also associated with prothrombotic complication with excess production of tissue factor and plasminogen activator inhibitor-1 [3,4]. Chronic low-grade inflammation is a key contributor to the initiation and development of obesity-related diseases [5], and is characterized by increased secretion of proinflammatory cytokines (TNF-α, IL-6) and decreased secretion of anti-inflammatory cytokines (adiponectin, IL-10) [6,7]. Macrophages are one of the major sources of the inflammatory responses [8], which are associated with high cell infiltration of expanding adipose tissue in obese individuals [7]. In addition to increased macrophage recruitment to adipose tissue, obesity polarizes anti-inflammatory macrophages (M2; alternatively activated macrophages) to proinflammatory macrophages (M1; classically activated macrophages) [9].

Free fatty acid (FFA) levels are elevated in obese subjects through release from enlarged adipose tissue or reduced clearance [10]. FFA promotes inflammatory response signaling through Toll-like receptor (TLR) 4 and activates NF-κB and/or JNK signaling pathways that lead to insulin resistance, hepatic steatosis, and atherosclerosis [6,11–13]. Attenuation of these inflammatory responses, by targeting NF-κB or JNK or neutralizing TNF-α and IL-1β, improves insulin resistance and fatty liver diseases [14–16]. In addition to activation of TLR4, it has been reported that FFA activates NLRP3 inflammasomes, leading to activation of caspase 1 and secretion of IL-1β and IL-18 [16].

Although chronic low-grade inflammation plays a role in obesity-related complications [1,5], little is known about the molecular mechanism underlying its occurrence during HFD intake. A recent study showed that histones, which are nuclear proteins, function as endogenous danger signals or alarmins when they are released into the extracellular space during ischemia-reperfusion injury [17]. Extracellular histones can bind to different immune receptors, TLR2, TLR4, and TLR9, and contribute to leukocytosis, endothelial

* Corresponding author. Address: Department of Systems Biology in Thromboregulation, Kagoshima University Graduate School of Medical and Dental Sciences, 8-35-1 Sakuragaoka, Kagoshima 890-8544, Japan. Fax: +81 99 275 6463.
E-mail address: rinken@m3.kufm.kagoshima-u.ac.jp (I. Maruyama).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.06.117

dysfunctions, organ failure, and even death [17–20]. In the present study, we show that HFD feeding or FFA treatment induces an active secretion of histone H3 into the extracellular space. This secretion is mediated, in part, through reactive oxygen species (ROS) production and the JNK signaling pathway. The extracellular histone then activates endothelial cells to express adhesion molecules, intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), and a procoagulant molecule, tissue factor. These findings suggest the possible contribution of extracellular histone to the pathogenesis of HFD-induced inflammation and thrombosis.

2. Materials and methods

2.1. Reagents

Sodium palmitate (P9767), FFA-free BSA (A6003), N-acetyl cysteine (NAC) (A9165), Bay-11 7082 and oleate (07501G) were obtained from Sigma–Aldrich (St Louis, MO, USA). SP600125 (FA-005) was purchased from SABioscience (Valencia, CA, Spain). SB203580 (559389) and U0126 (662005) were obtained from Calbiochem (Billerica, MA, USA). Anti-histone H3 antibodies, sc-8654 and ab1791, were purchased from Santa Cruz Biotechnology (Dallas, TX, USA) and Abcam (Cambridge, MA, UK), respectively. Antibodies against p-SAPK/JNK, p-p42/44, p-p38, and p-p65 were purchased from Cell Signaling Technology (Beverly, MA, Canada).

2.2. Palmitate/BSA complex solution preparation

The palmitate/BSA solution was prepared as described previously [21] with slight modifications. Briefly, 100 mM palmitate solution was solubilized in 50% ethanol and then conjugated with 5% FFA-free BSA to achieve a final palmitate concentration of 5 mM. The conjugation was performed to increase the solubility of palmitate. The corresponding BSA solution with ethanol was used as a control. The solutions were prepared on the same day of the experiments.

2.3. Cell culture and treatment

A murine macrophage cell line, RAW 264.7, was obtained from the American Type Culture Collection (Manassas, VA, USA). The cells were cultured in RPMI-1640 medium (Sigma Chemical Co., St. Louis, MO, USA) supplemented with 10% FBS (Hyclone, Logan, UT, USA), 100 U/ml penicillin–streptomycin, and 5 mg/L amphotericin. The cells were maintained at 37 °C under 5% CO_2. The cells were starved for 2 h in serum-free medium and then stimulated with palmitate/BSA (Pa–BSA) or BSA solution as indicated. Pretreatments with different inhibitors were carried out 1 h before cell stimulation.

2.4. Sample preparation for histone H3

The histone H3 levels in culture supernatants were analyzed by Western blotting as described previously [22]. Briefly, 1.6 ml of supernatant was incubated with heparin-Sepharose 6B beads (GE Healthcare Bio-Science, Piscataway, NJ) at 4 °C overnight, washed with PBS, and mixed with sample buffer for SDS–PAGE.

2.5. Western blotting

Cell lysates or prepared samples were separated by 10–15% SDS–PAGE, transferred to nitrocellulose membranes, blocked with 5% nonfat dry milk, and incubated with specific primary antibodies against histone H3 (1:500; Santa Cruz Biotechnology), p-SAPK/JNK (1:1000), p-p42/p44 (1:1000), p-p38 (1:1000), p-p65 (1:1000) and β-actin (1:1000; Santa Cruz Biotechnology) at 4 °C overnight. The membranes were then incubated with horseradish peroxidase-conjugated secondary antibodies (1:3000; MP Biomedicals, L.L.C, Irvine, CA, USA) followed by detection with an ECL detection system (Thermo Scientific, Waltham, MA, USA). The band intensities were measured using ImageJ 1.46 software (National Institutes of Health, Bethesda, MD, USA).

2.6. Immunocytochemistry

RAW 264.7 cells plated on four-chamber culture slides for 16 h were fixed with 2% paraformaldehyde, permeabilized with a mixture of ethanol and acetone (2:1) at –20 °C for 5 min, and blocked with 1% BSA in PBS containing 0.1% Triton X-100. The cells were then sequentially incubated with an anti-histone H3 antibody (1:250; Abcam) for 24 h at 4 °C, and an Alexa Fluor 488-labeled goat anti-rabbit secondary antibody (A11070; Life Technologies, Carlsbad, CA, USA) for 1 h. The nuclei were stained with DAPI. The stained cells were visualized and photographed under a confocal microscope (LSM700; Carl Zeiss, Oberkochen, Germany).

2.7. Immunohistochemical staining

Immunohistochemical staining of histone H3 was performed as described previously [23] with slight modifications. Briefly, epididymal adipose tissue samples from male C57BL/6J mice fed a normal chow diet (crude fat: 4.6%, CLEA Japan Inc., Tokyo, Japan) or HFD-60 (crude fat: 35%, Oriental Yeast Co., Tokyo, Japan) for 12 weeks, were fixed in 10% formaldehyde neutral buffer, paraffin-embedded, and sectioned. The tissue sections were deparaffinized and rehydrated. Endogenous peroxidase activity was blocked in the presence of 0.3% hydrogen peroxide in methanol for 15 min. After antigen retrieval using an antigen-unmasking solution (Vector Laboratories Inc., Burlingame, CA, USA), the sections were blocked with 1% BSA in PBS containing 0.01% Tween 20 for 1 h. Subsequently, the sections were incubated with a rabbit anti-histone H3 antibody (1:800; Abcam) for 30 min at room temperature, followed by incubation with a secondary antibody for 30 min using a Histofine Simple Stain Mouse MAX-PO Kit (Nichirei Co., Tokyo, Japan). Finally, the sections were stained with DAB and counterstained with hematoxylin. Photographs were taken using a Zeiss Axiophot microscope (Carl Zeiss, Oberkochen, Germany).

2.8. ROS measurement

Intracellular ROS production was detected using the fluorescent probe H_2DCFDA (C400; Life Technologies) as described previously [24]. Briefly, RAW 264.7 cells treated with palmitate for 8 h were washed and incubated with 10 μM H_2DCFDA for 45 min at 37 °C. The fluorescence signals were analyzed using an Epics XL flow cytometer (Beckman Coulter, Miami, FL, USA).

2.9. Total RNA isolation and quantitative real-time RT-PCR

Human umbilical vein endothelial cells (HUVECs) were treated with purified unfractionated calf thymus histone (Sigma–Aldrich, St. Louis, MO, USA) at concentrations of 0, 10 and 20 μg/ml for 4 h. Total RNA was extracted from HUVECs using an RNAqueous Total RNA Isolation Kit (Ambion, Austin, TX, USA) according to the manufacturer's protocol, and reverse-transcribed to cDNA. The mRNA expression levels of inflammatory genes were assessed with specific primers (Assay IDs: tissue factor, Hs01076029_m1; ICAM-1, Hs00164932_m1; VCAM-1, Hs003655486_m1; TLR 2, Hs00610101_m1 and TLR 4, Hs00370853_m1) as described previously [23]. Expression levels were calculated as the ratio of mRNA

level for a given gene relative to the mRNA level for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the same cDNA sample.

2.10. Flow cytometric assay of tissue factor, ICAM-1 and VCAM-1

The cell surface expressions of tissue factor protein, ICAM-1 and VCAM-1 protein were assessed by flow cytometry as described previously [25] using fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies against human tissue factor (Sekisui Diagnostics), ICAM-1 (Beckman Coulter, Marseille, France) and VCAM-1 (BD Pharmingen).

2.11. Cell viability assay

Cell viability was assessed by MTT assays as described previously [22]. Briefly, 5×10^5 cells in 24-well plates were treated with different concentrations of palmitate (0–200 μM) for 16 h. The cells were then incubated with MTT solution for 3 h. The formazan product was solubilized in dimethyl sulfoxide, and the absorbances were measured at a wavelength of 570 nm with a reference wavelength of 630 nm.

2.12. Statistical analysis

All results are expressed as means ± SEM, and were statistically analyzed by one-way ANOVA or a *t*-test using Prism software. Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Extranuclear release of histone H3 in HFD-fed mice

Histones are found in the nucleus of resting cells where they regulate transcription and are involved in chromatin remodeling [18,19]. Consistent with this, histone H3 was mainly localized in the cell nuclei in mice fed the normal chow (Fig. 1A, left panel). However, extranuclear histone H3 was increased in mice fed the HFD (Fig. 1A, right panel). An increase in extranuclear histone H3 was also observed in murine macrophage cell line RAW 264.7 cells treated with the saturated fatty acid palmitate (Fig. 1B). These results indicate that HFD feeding or saturated fatty acid treatment can induce translocation of histone H3 from the nucleus to the cytoplasm.

3.2. Saturated fatty acid palmitate induces extracellular release of histone H3 from RAW 264.7 cells

We treated RAW 264.7 cells with palmitate for time periods of 8–16 h and at increasing concentrations (0–200 μM) for 16 h. Palmitate induced extracellular release of histone H3 in 16 h (Fig. 2A) and in a dose-dependent manner (Fig. 2B). In contrast, the carrier protein BSA or unsaturated fatty acid oleate did not induce histone H3 release (Fig. 2C). The palmitate-induced histone H3 release was not caused by cell death (Fig. 2D), suggesting that active secretion, rather than passive leakage, may be involved in this process.

3.3. ROS and JNK signaling mediate palmitate-induced histone H3 release

Next, we examined the mechanism by which palmitate induces release of histone H3. In line with previous reports [16,26], palmitate induced ROS generation in RAW 264.7 cells (Fig. 3A). Treatment with NAC, a ROS inhibitor, attenuated ROS production (Fig. 3A) and inhibited palmitate-induced histone H3 release (Fig. 3B). These results indicate that palmitate-induced histone H3 release is mediated, in part, through ROS production. We further examined the signaling pathways for histone H3 release. As shown in Fig. 3C, palmitate increased the phosphorylation of p38, JNK, ERK1/2, and NF-κB p65. A selective inhibitor of JNK (SP600125) attenuated palmitate-induced histone H3 release,

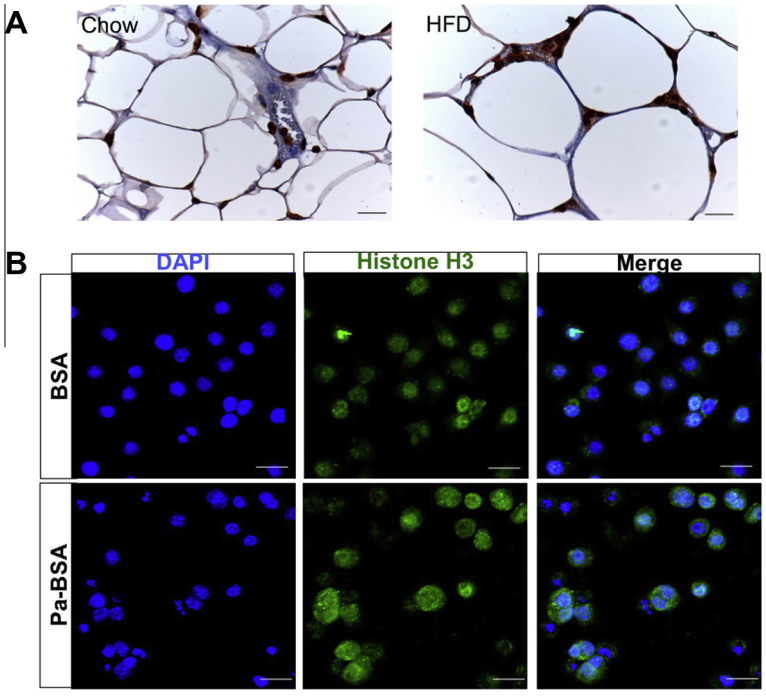

Fig. 1. Nuclear translocation of histone H3 *in vitro* and *in vivo*. (A) Representative photographs of immunostaining for histone H3 in adipose tissue from mice fed with a normal diet or HFD for 12 weeks. Original magnification: 40×; scale bar: 20 μm. (B) RAW 264.7 cells were plated on four-chamber culture slides and treated with 200 μM of BSA or palmitate/BSA (Pa-BSA) for 16 h. The cells were then immunostained for histone H3 and the nuclei were counterstained with DAPI. Original magnification: 20×; scale bar: 20 μm.

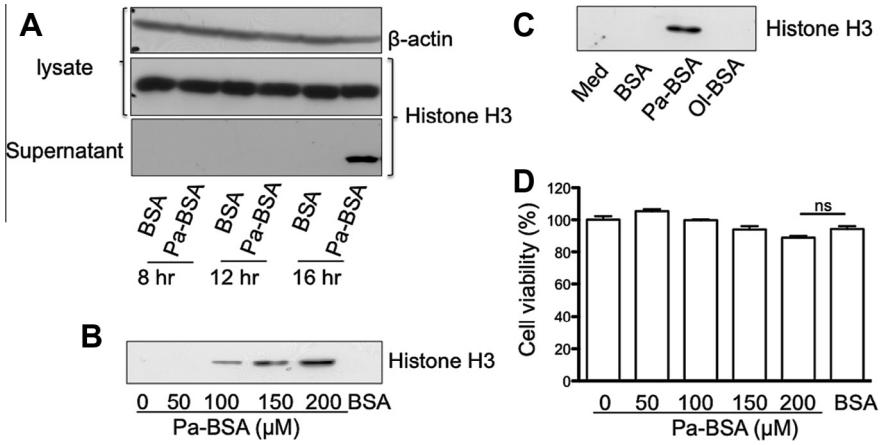

Fig. 2. Palmitate induces histone H3 release in time- and dose-dependent manners from RAW 264.7 cells. (A) RAW 264.7 cells were incubated with 200 μM of BSA or palmitate/BSA (Pa–BSA) for 8–16 h and histone H3 levels in supernatants and cell lysates were analyzed by Western blotting. (B) RAW 264.7 cells were incubated with increasing concentrations of Pa–BSA (0–200 μM) for 16 h and histone H3 levels in supernatants were analyzed by Western blotting. (C) RAW 264.7 cells were treated with 200 μM of BSA, Pa–BSA, or oleate/BSA (Ol-BSA) for 16 h, and histone H3 levels in supernatants were analyzed by Western blotting. (D) Cell viability was measured by MTT assays after 16 h of palmitate incubation.

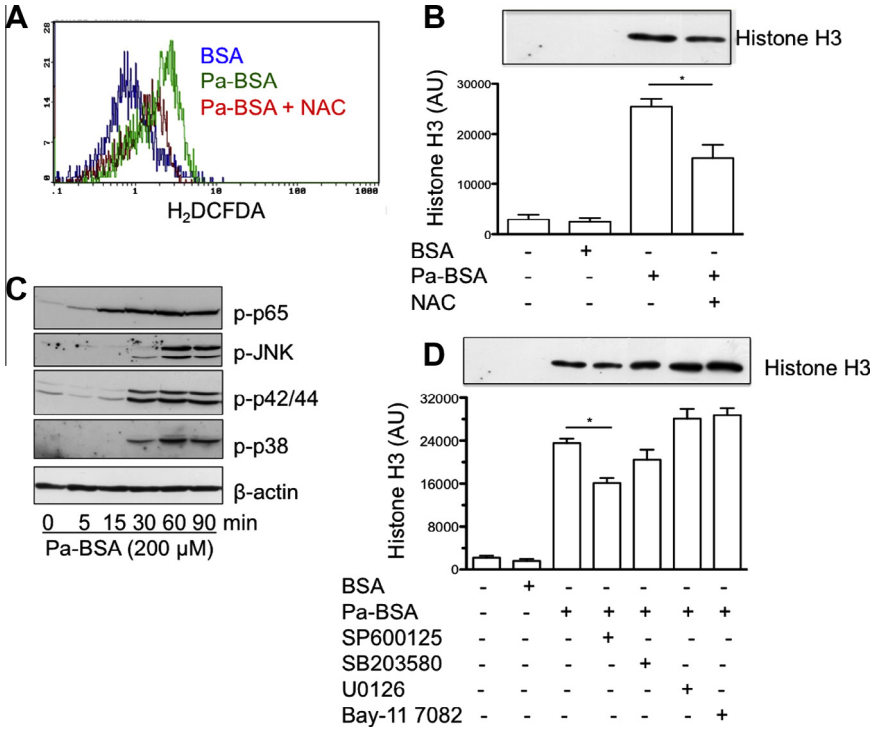

Fig. 3. Palmitate induces histone H3 through ROS and JNK signaling. (A) RAW 264.7 cells were treated with 200 μM of BSA or palmitate/BSA (Pa-BSA) for 8 h. Intracellular ROS production was measured using a fluorescent probe, H_2DCFDA. (B) RAW 264.7 cells were pretreated with NAC (5 mM) 1 h before palmitate stimulation. The histone H3 levels in the supernatants after 16 h were analyzed by Western blotting. (C) Immunoblotting for phospho-MAPKs and phospho-p65 in lysates of cells treated with palmitate (200 μM) for 0–90 min. (D) RAW 264.7 cells were pretreated with MAPK inhibitors (SB203580, SP600125, or U0126; 10 μM) and NF-κB inhibitors (10 μM) 1 h before cell stimulation. The histone H3 levels in the supernatants after 16 h were analyzed by Western blotting.

whereas a p38 inhibitor (SB203580), ERK1/2 inhibitor (U0126), and NF-κB inhibitor (Bay-11 7082) had no effects (Fig. 3B). These results indicate that palmitate-induced histone H3 release is mediated, in part, through the JNK signaling pathway.

3.4. Extracellular histone activates endothelial cells to express the adhesion molecules VCAM-1 and ICAM-1 and the procoagulant molecule tissue factor

Finally, we examined the effects of extracellular histone on the surrounding cells, including endothelial cells. Extracellular histone significantly increased the mRNA expressions of the adhesion molecules ICAM-1 and VCAM-1 and the procoagulant molecule tissue factor in endothelial cells in dose-dependent manners (Fig. 4A). Extracellular histone also increased the mRNA expression of TLR2, but not that of TLR4 (Fig. 4A), which were both reported to act as receptors for extracellular histones [18–20]. Furthermore, extracellularhistone induced the expression of ICAM-1, VCAM-1, and tissue factor proteins on the surface of endothelial cells (Fig. 4B). These results suggest a possible contribution of extracellular histones to the pathogenesis of HFD-induced inflammation and thrombosis.

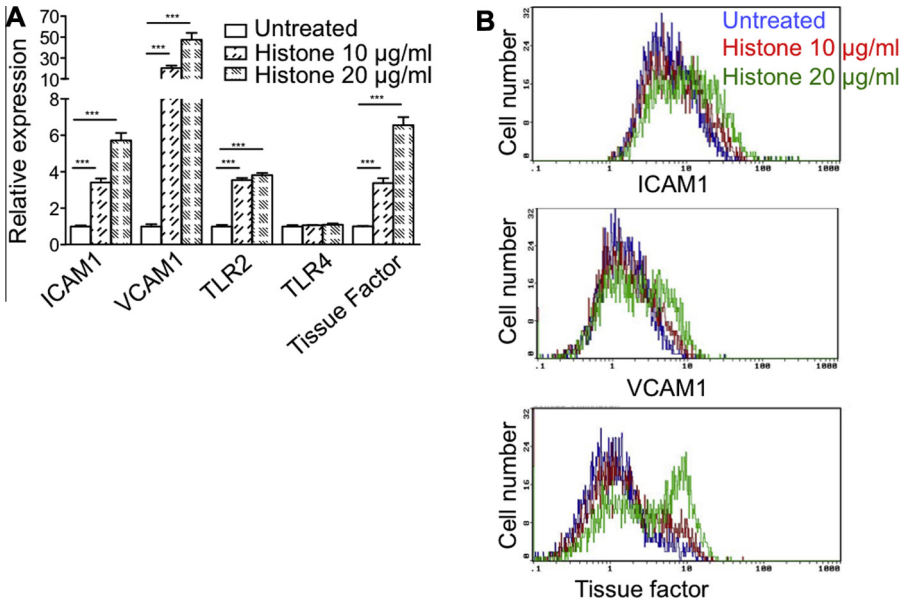

Fig. 4. Extracellular histone induces the expressions of the adhesion molecules ICAM-1 and VCAM-1 and the procoagulant molecule tissue factor in endothelial cells. (A) HUVECs were treated with histone at concentrations of 0, 10 and 20 μg/ml for 4 h, and the expressions of ICAM-1, VCAM-1, TLR2, TLR4 and tissue factor were analyzed by RT-PCR. (B) HUVECs were treated with histone for 6 h and measured for their surface expression of ICAM-1, VCAM-1 and tissue factor by flow cytometry.

4. Discussion

The present study has demonstrated that histone H3 is released into the extracellular space from macrophages treated with palmitate *in vitro* and adipose tissue in mice fed a HFD *in vivo*. The release of histone H3 is an active process in living cells, rather than passive leakage from dying cells. The extracellular histone then activates the endothelium to express cell adhesion molecules and procoagulant protein, tissue factor. These results suggest the possible contribution of extracellular histone to the pathogenesis of HFD-induced inflammation and thrombosis.

Histones are nuclear proteins that form hetero-octamers to wind up the double strands of DNA in nucleosomes and are involved in chromatin remodeling and gene transcription regulation [18,19]. Histones can be released into the extracellular space during sepsis, and the plasma concentration of histones can reach 70 μg/ml in the acute inflammatory condition [27]. In this study, we showed that histone H3 was also released into the extracellular space during chronic low-grade inflammation induced by HFD feeding or FFA treatment. Palmitate (C16:0) and oleate (C18:1) are most abundant saturated and unsaturated fatty acid, respectively, in HFD used in this study. Consistent with previous studies showing that palmitate induces expression of proinflammatory molecules [6,28,29], palmitate, but not oleate, induced histone H3 release in our *in vitro* study.

ROS are linked to multiple pathologies, such as cardiovascular diseases, diabetes, neurological disease, and cancer. The increased ROS levels during obesity deregulate the production of adipokines [30]. Consistent with previous data, we found that palmitate induced ROS generation from RAW 264.7 cells, and that treatment with NAC, a ROS inhibitor, inhibited the histone release. In addition to ROS, NF-κB and JNK, which are regulators of inflammation, have been reported to link obesity and metabolic diseases [12]. FFA activates both NF-κB and JNK signaling following activation of TLR4, leading to increased expressions of proinflammatory cytokines such as TNF-α and IL-6 [6]. Our results also demonstrated activation of NF-κB and MAPKs in time-dependent manners. Although palmitate activated these various pathways, incubation of the cells with ERK, p-38, and NF-κB inhibitors in presence of palmitate had no effects on the histone release. However, a JNK inhibitor inhibited the histone release. These results suggest that the JNK signaling pathway is involved in the histone release induced by palmitate.

A previous study showed that the JNK signaling pathway is activated by palmitate, and that inhibition of ROS reduces the activation of this signaling pathway and enhances insulin sensitivity in hepatocytes [26]. We treated cells with palmitate in the presence of NAC or a JNK inhibitor and measured the activation of JNK by Western blotting. The cells treated with the JNK inhibitor showed attenuated JNK activation, while NAC did not inhibit the JNK activation (data not shown). Our results show that the activation of JNK is not dependent on ROS and that both ROS and JNK activation partly contribute to the histone release induced by palmitate.

Finally, we performed *in vitro* experiments examining the effects of the extracellular histone. It has been reported that histones, which are released from dying cells during sepsis or inflammation, can activate TLR2/TLR4 and contribute to endothelial dysfunction [19]. ICAM-1 and VCAM-1 are upregulated during endothelial activation and involved in the interactions of inflammatory cells with the endothelium and the transmigration of these cells into adipose tissue [31]. We showed that the released histone induced the expressions of adhesion molecules ICAM-1 and VCAM-1 in endothelial cells and enhanced the expression of TLR2, but not TLR4. Besides these effects, the histone induced the expression of a procoagulant protein, tissue factor, from endothelial cells, which plays a crucial role in coagulation and thrombosis. A recent study showed that tissue factor promotes inflammation and diet-induced obesity through protease-activated receptor 2 (PAR2) [32].

In summary, our study has demonstrated that palmitate induces the release of histone H3 from activated macrophages, in part, through ROS generation and the JNK signaling pathway. The extracellular histone activates the endothelium and enhances proinflammatory and prothrombotic responses. Overall, we suggest that histones provide the cross-talk between obesity and inflammation. However, further studies are required to investigate the associations of histones with diet-induced obesity and inflammation.

Acknowledgments

We thank Nobue Uto (Department of Laboratory and Vascular Medicine), Tomoka Nagasato, and Mika Yamamoto (Department of Systems Biology in Thromboregulation) for their technical support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.06.117.

References

[1] T.D. Kanneganti, V.D. Dixit, Immunological complications of obesity, Nat. Immunol. 13 (2012) 707–712.
[2] J.O. Hill, Understanding and addressing the epidemic of obesity: an energy balance perspective, Endocr. Rev. 27 (2006) 750–761.
[3] A. Singh, G.D. Foster, J. Gunawardana, T.A. McCoy, T. Nguyen, S. Vander Veur, E. Komaroff, A.K. Rao, Elevated circulating tissue factor procoagulant activity, factor VII, and plasminogen activator inhibitor-1 in childhood obesity: evidence of a procoagulant state, Br. J. Haematol. 158 (2012) 523–527.
[4] K.A. Darvall, R.C. Sam, S.H. Silverman, A.W. Bradbury, D.J. Adam, Obesity and thrombosis, Eur. J. Vasc. Endovasc. Surg. 33 (2007) 223–233.
[5] C.N. Lumeng, A.R. Saltiel, Inflammatory links between obesity and metabolic disease, J. Clin. Invest. 121 (2011) 2111–2117.
[6] H. Shi, M.V. Kokoeva, K. Inouye, I. Tzameli, H. Yin, J.S. Flier, TLR4 links innate immunity and fatty acid-induced insulin resistance, J. Clin. Invest. 116 (2006) 3015–3025.
[7] S.P. Weisberg, D. McCann, M. Desai, M. Rosenbaum, R.L. Leibel, A.W. Ferrante Jr., Obesity is associated with macrophage accumulation in adipose tissue, J. Clin. Invest. 112 (2003) 1796–1808.
[8] H. Xu, G.T. Barnes, Q. Yang, G. Tan, D. Yang, C.J. Chou, J. Sole, A. Nichols, J.S. Ross, L.A. Tartaglia, H. Chen, Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance, J. Clin. Invest. 112 (2003) 1821–1830.
[9] C.N. Lumeng, J.L. Bodzin, A.R. Saltiel, Obesity induces a phenotypic switch in adipose tissue macrophage polarization, J. Clin. Invest. 117 (2007) 175–184.
[10] G. Boden, Obesity and free fatty acids, Endocrinol. Metab. Clin. North Am. 37 (vii–ix) (2008) 635–646.
[11] J. Nigro, N. Osman, A.M. Dart, P.J. Little, Insulin resistance and atherosclerosis, Endocr. Rev. 27 (2006) 242–259.
[12] G. Solinas, M. Karin, JNK1 and IKKbeta: molecular links between obesity and metabolic dysfunction, FASEB J. 24 (2010) 2596–2611.
[13] L. Li, L. Chen, L. Hu, Y. Liu, H.Y. Sun, J. Tang, Y.J. Hou, Y.X. Chang, Q.Q. Tu, G.S. Feng, F. Shen, M.C. Wu, H.Y. Wang, Nuclear factor high-mobility group box1 mediating the activation of Toll-like receptor 4 signaling in hepatocytes in the early stage of nonalcoholic fatty liver disease in mice, Hepatology 54 (2011) 1620–1630.
[14] M.C. Arkan, A.L. Hevener, F.R. Greten, S. Maeda, Z.W. Li, J.M. Long, A. Wynshaw-Boris, G. Poli, J. Olefsky, M. Karin, IKK-beta links inflammation to obesity-induced insulin resistance, Nat. Med. 11 (2005) 191–198.
[15] M.S. Han, D.Y. Jung, C. Morel, S.A. Lakhani, J.K. Kim, R.A. Flavell, R.J. Davis, JNK expression by macrophages promotes obesity-induced insulin resistance and inflammation, Science 339 (2013) 218–222.
[16] H. Wen, D. Gris, Y. Lei, S. Jha, L. Zhang, M.T. Huang, W.J. Brickey, J.P. Ting, Fatty acid-induced NLRP3-ASC inflammasome activation interferes with insulin signaling, Nat. Immunol. 12 (2011) 408–415.
[17] H. Huang, J. Evankovich, W. Yan, G. Nace, L. Zhang, M. Ross, X. Liao, T. Billiar, J. Xu, C.T. Esmon, A. Tsung, Endogenous histones function as alarmins in sterile inflammatory liver injury through Toll-like receptor 9 in mice, Hepatology 54 (2011) 999–1008.
[18] R. Allam, C.R. Scherbaum, M.N. Darisipudi, S.R. Mulay, H. Hagele, J. Lichtnekert, J.H. Hagemann, K.V. Rupanagudi, M. Ryu, C. Schwarzenberger, B. Hohenstein, C. Hugo, B. Uhl, C.A. Reichel, F. Krombach, M. Monestier, H. Liapis, K. Moreth, L. Schaefer, H.J. Anders, Histones from dying renal cells aggravate kidney injury via TLR2 and TLR4, J. Am. Soc. Nephrol. 23 (2012) 1375–1388.
[19] F. Semeraro, C.T. Ammollo, J.H. Morrissey, G.L. Dale, P. Friese, N.L. Esmon, C.T. Esmon, Extracellular histones promote thrombin generation through platelet-dependent mechanisms: involvement of platelet TLR2 and TLR4, Blood 118 (2011) 1952–1961.
[20] J. Xu, X. Zhang, M. Monestier, N.L. Esmon, C.T. Esmon, Extracellular histones are mediators of death through TLR2 and TLR4 in mouse fatal liver injury, J. Immunol. 187 (2011) 2626–2631.
[21] S.P. Cousin, S.R. Hugl, C.E. Wrede, H. Kajio, M.G. Myers Jr., C.J. Rhodes, Free fatty acid-induced inhibition of glucose and insulin-like growth factor I-induced deoxyribonucleic acid synthesis in the pancreatic beta-cell line INS-1, Endocrinology 142 (2001) 229–240.
[22] Y. Nawa, K. Kawahara, S. Tancharoen, X. Meng, H. Sameshima, T. Ito, Y. Masuda, H. Imaizumi, T. Hashiguchi, I. Maruyama, Nucleophosmin may act as an alarmin: implications for severe sepsis, J. Leukoc. Biol. 86 (2009) 645–653.
[23] B. Shrestha, T. Hashiguchi, T. Ito, N. Miura, K. Takenouchi, Y. Oyama, K. Kawahara, S. Tancharoen, I.Y. Ki, N. Arimura, N. Yoshinaga, S. Noma, C. Shrestha, T. Nitanda, S. Kitajima, K. Arimura, M. Sato, T. Sakamoto, I. Maruyama, B cell-derived vascular endothelial growth factor A promotes lymphangiogenesis and high endothelial venule expansion in lymph nodes, J. Immunol. 184 (2010) 4819–4826.
[24] M.Y. Chang, C.Y. Han, T.N. Wight, A. Chait, Antioxidants inhibit the ability of lysophosphatidylcholine to regulate proteoglycan synthesis, Arterioscler. Thromb. Vasc. Biol. 26 (2006) 494–500.
[25] T. Ito, K. Kawahara, T. Nakamura, S. Yamada, K. Abeyama, T. Hashiguchi, I. Maruyama, High-mobility group box 1 protein promotes development of microvascular thrombosis in rats, J. Thromb. Haemost. 5 (2007) 109–116.
[26] S. Nakamura, T. Takamura, N. Matsuzawa-Nagata, H. Takayama, H. Misu, H. Noda, S. Nabemoto, S. Kurita, T. Ota, H. Ando, K. Miyamoto, S. Kaneko, Palmitate induces insulin resistance in H4IIEC3 hepatocytes through reactive oxygen species produced by mitochondria, J. Biol. Chem. 284 (2009) 14809–14818.
[27] J. Xu, X. Zhang, R. Pelayo, M. Monestier, C.T. Ammollo, F. Semeraro, F.B. Taylor, N.L. Esmon, F. Lupu, C.T. Esmon, Extracellular histones are major mediators of death in sepsis, Nat. Med. 15 (2009) 1318–1321.
[28] P.S. Laine, E.A. Schwartz, Y. Wang, W.Y. Zhang, S.K. Karnik, N. Musi, P.D. Reaven, Palmitic acid induces IP-10 expression in human macrophages via NF-kappaB activation, Biochem. Biophys. Res. Commun. 358 (2007) 150–155.
[29] J.Y. Lee, K.H. Sohn, S.H. Rhee, D. Hwang, Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2 mediated through Toll-like receptor 4, J. Biol. Chem. 276 (2001) 16683–16689.
[30] S. Furukawa, T. Fujita, M. Shimabukuro, M. Iwaki, Y. Yamada, Y. Nakajima, O. Nakayama, M. Makishima, M. Matsuda, I. Shimomura, Increased oxidative stress in obesity and its impact on metabolic syndrome, J. Clin. Invest. 114 (2004) 1752–1761.
[31] S.E. Shoelson, J. Lee, A.B. Goldfine, Inflammation and insulin resistance, J. Clin. Invest. 116 (2006) 1793–1801.
[32] L. Badeanlou, C. Furlan-Freguia, G. Yang, W. Ruf, F. Samad, Tissue factor-protease-activated receptor 2 signaling promotes diet-induced obesity and adipose inflammation, Nat. Med. 17 (2011) 1490–1497.